## REMARKS

Claims 1-21 are currently pending in the application. Claims 2-3, and 7-21 are withdrawn. Claims 5 and 6 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

### Claim Objections

The Examiner has objected to claim 5 as containing a misspelling. Applicants have corrected this error, as well as an additional spelling error identified in claim 6. Withdrawal of the objection is hereby respectfully requested.

### Rejection of Claims 1 and 4-6 Under 35 U.S.C. §103(a)

The Examiner has rejected claims 1 and 4-6 under 35 U.S.C. §103(a) as obvious over the teachings of Shuman (U.S. Pat. No. 5,766,891) in view of Heyman et al. (Genome Research, 9:383-392, 1999), Pan et al. (J. Biol. Chem. 225: 890-901, 1993) and in view of Sambrook et al. (Molecular Cloning pp13-15, 1982). Applicants respectfully disagree.

The Examiner asserts that the claims are drawn to

> "methods of covalently linking or cloning nucleic acid inserts to nucleic acid flanking molecules, which nucleic acids comprise covalently linked topoisomerase polypeptides, and which linkage method may further comprise treatment of ligated nucleic acid inserts with phosphatase for further ligation, whereby ligation occurs via a ligase, topoisomerase, by site-specific or homologous recombination, or alternatively the nucleic acid inserts are ligated into a circular cloning vector and subsequently transformed into an appropriate host cell".

Applicant submits that, as claimed, the elected invention is drawn to methods for molecular cloning wherein an insert molecule having a 5'-hydroxyl group at each end is incubated with a first and second flanking molecule having a topoisomerase bound to only one end of each of the first and second flanking molecules, under conditions which permit covalent

joining of the insert and the first and second flanking molecules, and transforming a host cell with the resulting molecule. The elected invention is also drawn to a method of molecular cloning wherein the insert molecule has a 5'-hydroxyl group at one end and a 5'- phosphate at the other end, and the insert is incubated with a linear cloning vector having a topoisomerase polypeptide at one end and a ligation substrate site at the other end, under conditions that permit covalent joining to form a circular vector. There is no step in the elected claims that requires the "treatment of ligated nuclei acid inserts with phosphatase for further ligation." Thus, the Examiner's reliance on the teachings of Sambrook relating to removal of phosphate residues is unclear, and appears to be misplaced. Further clarification is respectfully requested.

The Examiner asserts that Shuman teaches methods of both non-directional and directional ligating/cloning of nucleic acids comprising linkers for topoisomerase mediated ligation of inserts with flanking nucleic acid molecules. The Examiner asserts that the flanking nucleic acid molecules include bound topoisomerase, ligation substrate sites and recombination sites. The Examiner asserts that Heyman et al. teach topoisomerase mediated high throughput cloning/ligation comprising the joining of nucleic acid fragments to plasmid vectors. The Examiner asserts that the nucleic acid fragments contain covalently bound topoisomerase and free 5'-hydroxyl termini. The Examiner notes that the primary references do not teach the removal of phosphate groups from the nucleic acid inserts using phosphatase, nor the use of recombinases for ligation, but that these aspects are provided by the teachings of Pan et al. and Sambrook et al. Again, the instant claims do not require the removal of a phosphate group prior to joining of the insert to the flanking molecules, and the Examiner's reliance on Pan et al and Sambrook et al. for this teaching is somewhat confusing. To the extent that the Examiner maintains this line of rejection in a subsequent office action, further clarification is respectfully requested.

For the reasons described below, the Examiner has failed to establish a *prima facie* case of obviousness under the requirements of 35 U.S.C. § 103(a). To establish a *prima facie* case of obviousness *inter alia*, the prior art reference (or references when combined) must teach or suggest *all the claim limitations*. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974). Applicants submit that the art cited by the Examiner, when considered alone or together fails to

teach each limitation of the claimed invention and, thus, does not render the instant claims obvious. The claims are distinguished over the cited prior art in at least the following general ways.

First, neither Shuman or Heyman et al., either individually or in combination, teaches the covalent joining of flanking nucleic acid molecules to an insert molecule, such that the insert molecule is positioned between first and second flanking molecules, and transformation of a host cell with the resulting construct as required, for example, by claim 1. The cited art uses linearized vectors that, with the single step of ligating an insert, result in circular vector constructs. The topoisomerase-mediated covalent joining of the claimed methods results in a linear molecule which requires another ligation event to form a covalently joined circular construct.

Second, neither Shuman or Heyman, either individually or in combination, teaches or suggests methods in which the insert molecule has a 5'-OH on one end and a 5'-phosphate on the other end, as required, for example, by claim 6. This requirement that the insert molecule have a 5'-OH on end and a 5'-phosphate on the other provides a directionality to the cloning that is not taught or suggested by the cited art. The claimed invention therefore does not require linker molecules as taught in Shuman. The only topoisomerase-mediated covalent joining method described in the cited art that has a directionality requires the use of linker molecules that will result in different overhangs when cleaved by restriction enzymes (Shuman, column 6, lines 33-35).

In each of Shuman and Heyman et al., when an insert is ligated between the ends of a linearized vector, either both ends of the insert or both ends of the vector are covalently bound to a topoisomerase. For example, the Examiner cites Figure 1 of Heyman as teaching nucleic acid fragments containing covalently bound topoisomerase and free 5'- hydroxyl termini. A review of figure 1, however, shows that it reports a method for cloning a PCR fragment, in which it is the fragment that contains the 5'-hydroxyl residues, but that the plasmid vector comprises topoisomerase on each of its free ends; that is, the vector does not have topoisomerase on one end only as required by the instant claims. With respect to the teachings of Shuman, there is no

teaching or suggestion of the use of flanking molecules with one end only bound to a topoisomerase polypeptide in a method of molecular cloning involving covalently joining a nucleic acid insert molecule to first and second flanking nucleic acid molecules or ends. For example, at column 5, line 39 to column 6, line 19 Shuman describes double stranded DNA molecules "having at *each end thereof the modified vaccinia topoisomerase enzyme,*" (column 5, lines 41 and 42; emphasis added) which clearly teaches topoisomerase at both ends of the molecule. In the same cited passage, there is the statement:

> "[I]n this method of ligation, the donor duplex DNA substrate is *a bivalent donor duplex DNA substrate, that is, it contains two topoisomerase cleavage sites.* One embodiment comprises cleaving a donor duplex DNA substrate containing sequence specific topoisomerase cleavage *sites* by incubating the donor duplex DNA substrate with a sequence specific topoisomerase to form a topoisomerase-bound donor duplex DNA strand and incubating the topoisomerase-bound donor duplex DNA strand with a 5' hydroxyl-terminated compatible acceptor DNA, resulting in ligation of the topoisomerase-bound donor duplex DNA strand to the DNA acceptor strand." (column 5, line 66 to column 6, line 10; emphasis added)

Applicants submit that the passage quoted above teaches only bivalent donor duplex DNA substrates with two topoisomerase cleavage sites. Reaction of such a substrate with topoisomerase will result in the duplex DNA strand having a topoisomerase polypeptide covalently joined at *each* site, not at one end only, as required by each of the subject claims.

Shuman also teaches at column 8, lines 18 to 37, the advantages of topoisomerase cloning in general, and specifically states "[b]y designing a plasmid polylinker such that CCCTT sites are situated in inverted orientation on either side of a restriction site, one can generate a linear vector with topoisomerase sites at both 3' ends." Applicants submit that the vector resulting from reaction of such a vector with topoisomerase will generate a vector molecule with topoisomerase bound at each end. This is not a teaching of a molecule with topoisomerase bound at one end only, as required by each of the subject claims. Thus, the references do not teach or suggest a method of molecular cloning that uses first and second flanking molecules

with one end only bound to a topoisomerase polypeptide as recited in claim 1. The references also do not teach or suggest the covalent joining of first and second separate flanking nucleic acid molecules to an insert that has a 5'-OH group, as recited in claim 1. Applicants wish to emphasize that not only do Shuman and Heyman et al. not teach or suggest a method of molecular cloning involving the joining of molecules with one end only covalently bound to a topoisomerase, but neither reference teaches or suggests the use of separate first and second flanking molecules that are joined to an insert molecule. Further, the references do not teach or suggest the step of transforming a cell with a ligated molecule comprising an insert positioned between a first and a second flanking molecule as recited in claim 1.

The deficiencies in the teachings of Shuman and Heyman are not remedied by the teachings of either Pan et al. or Sambrook et al. Pan et al. do not teach or suggest molecular cloning at all, nor do they teach or suggest the covalent joining of flanking nucleic acid molecules with an insert to form a ligated molecule. The Pan et al. reference teaches the sealing of a single-strand nick on a double stranded DNA molecule by a topoisomerase. There is no teaching or suggestion that the property that allows sealing of a nick can be exploited to covalently join first and second flanking nucleic acid molecules, as recited by claim 1. In fact, Pan et al., which is concerned with elucidating the mechanisms of action of recombinases, does not teach or suggest any method of joining separate (e.g., flanking and insert) nucleic acid molecules. Because there is no teaching of covalently joining first and second flanking nucleic acid molecules with an insert, this reference also provides no teaching or suggestion of a molecular cloning method, particularly not a method involving transformation of a host cell, as required by claim 1.

Sambrook et al., relied on for teaching removal of phosphate residues by a phosphatase, does not teach or suggest a method of molecular cloning wherein first and second nucleic acid flanking molecules comprising covalently bound topoisomerase on one end only are covalently joined to an insert molecule. Applicants submit, therefore, that the cited references, whether considered alone or in combination, do not teach or suggest each of the elements of the claimed invention and, thus, the instant claims are not obvious over the references cited.

With particular respect to claim 6, as noted above, a key distinction over the cited art is the requirement that the insert molecule has a 5'-OH on one end and a 5'-phosphate on the other end, resulting in control over the directionality of the insert. The Heyman et al. reference does not address directionality at all. The Shuman reference only teaches that directionality can be controlled "by using a bivalent linker containing different overhangs at each cleavage site." Pan et al. does not teach or suggest cloning, directional or otherwise. Sambrook et al. do not teach directional cloning methods wherein the insert has a 5'-OH on one end and a 5'-phosphate on the other, and especially not in a method involving flanking nucleic acid molecules having a covalently bound topoisomerase molecule on one end only. Therefore, Applicants submit that each of the claims reciting this limitation cannot be obvious over any combination of the cited references.

In view of the above, Applicants submit that each of claims 1 and 4-6 is distinguished as non-obvious over the cited prior art. However, an additional reason Applicants disagree with the obviousness rejections relates to the Examiner's rationale for motivation to combine the teachings of the cited references. The Examiner's reasoning essentially equates to asserting that one of skill in the art would have been motivated to combine the references because the references allegedly teach the components of the invention. Not only do the references not provide all components necessary, as detailed above, but Applicants respectfully submit that it is a misapplication of the law to conclude that, because a collection of references allegedly teaches all necessary components of a claimed invention, the claimed invention is obvious. There must be a motivation provided, within the references themselves or in the knowledge generally available to one skilled in the art, to combine those components *in the manner claimed. In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

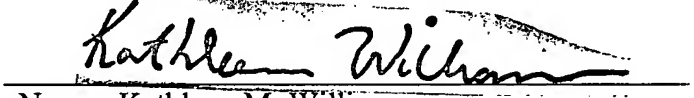
Applicants submit that neither Shuman, Heyman et al. nor Pan et al. teaches a method of cloning by covalently joining an insert molecule to first and second flanking molecules or ends of molecules wherein one end only of each of the first and second flanking molecules comprises a covalently bound topoisomerase polypeptide and where the insert has a 5'-OH on one end and a 5'-phosphate on the other and/or there is a transformation step to obtain transformants. Likewise, neither Shuman, Heyman et al. nor Pan et al. teach a method of cloning by joining an

insert molecule comprising a 5'-hydroxyl group at one end and a 5'-phosphate group at the other end with a linear cloning vector having a topoisomerase polypeptide at one end only and a ligation substrate site at the other end. The secondary references (i.e., Sambrook et al.) do not remedy this deficiency. The references themselves do not provide a motivation to combine the "components" in the manner claimed. Further, the Office Action provides no rationale whereby the general knowledge in the art can be said to provide such motivation. Therefore, applicants submit that even if the references do teach all elements of the claimed invention, which they do not, without the motivation to combine, it is improper to combine them for the purpose of the present obviousness rejection.

In view of the foregoing, Applicants submit that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: 9/2/05



Name: Kathleen M. Williams
Registration No.: 34,380
Customer No.: 27495
Palmer & Dodge LLP
111 Huntington Avenue
Boston, MA 02199-7613
Tel. (617) 239-0100